INSECT TRANSMISSION OF INFECTIOUS ANEMIA OF HORSES *

C. W. Howard

Swamp fever, or infectious anemia of horses, has been known in Minnesota for some years. At one time it was epidemic and while not at the present time taking that form, is probably more widely prevalent than suspected, because of the ability of the disease to assume a mild, unrecognized form.

The disease is widely distributed, having been recognized in Europe, Japan, recently by Theiler in South Africa, Panama Canal Zone, Canada, and in the United States from Wisconsin, Minnesota, North Dakota, Wyoming, Nevada, Washington, Colorado, Oklahoma, Nebraska, Missouri, Arkansas, and Mississippi.

The name would indicate that it was a disease of low lying, wet land. While this is, generally speaking, true, it is not always so. The European investigators particularly do not emphasize this fact, but have shown that horses pastured near forests are more subject to attack. The disease has been found at an elevation of 7,500 feet, and in many places where the soil was for the most part light and dry. In Minnesota most of the cases seem to have come either from the Red River Valley where the land is mostly low and level, of the prairie type, and the soil rather heavy, there being many swampy areas, or from the northern, undeveloped part of the state where there is much virgin timber and unreclaimed bogs and marshes. But other foci of the disease have been found in parts of the state where the land is almost entirely under cultivation with very little if any forest, and the soil of a light sandy nature. The swamps in these latter places are much restricted, and comparatively few in numbers, but still some are present.

The disease usually appears in early summer, increasing from July until October, and being recognized most widely during August and September. The incubation period seems to vary from ten to forty-five days, the usual time being twelve to fourteen days.

No other animals than equines seem to be susceptible. The Japanese investigators have claimed that the pig is slightly susceptible, and that young goats and sheep show slight febrile reactions, but other workers have not confirmed these results. This inability to employ any of the smaller laboratory animals has considerably hampered investigations, especially on the etiology of swamp fever.

---

* Paper No. 77—Journal Series, University of Minnesota Experiment Station.

NATURE OF THE VIRUS

The cause is an ultra-microscopic, filterable virus present in the blood. This may be transmitted to other horses by injections of blood serum which has been passed thru a filter. There are few, if any, symptoms specific for this disease, making it very difficult to recognize with complete positiveness, except by subinoculations of blood. This, together with the fact that only equines are susceptible and the impossibility of knowing when a horse may have had a mild attack of the disease and be immune to further infections has been a severe handicap in prosecuting investigations upon methods of transmission.

The virus seems to easily withstand freezing, but heating to 58° to 60° C. for one hour destroys it, as does bright sunlight. Drying at room temperature in a vacuum does not alter its virulence.

SYMPTOMS OF THE DISEASE

The most characteristic symptom is a progressive anemia without any apparent cause. This is accompanied by a recurrent fever at which time the temperature may rise to 103° F. or higher, dropping nearly or quite to normal after a short run. If worked, the horse tires very easily and may later develop a staggering gait. It may improve for a time only to be followed by a more severe attack. Death may result, or the animal may recover, under which circumstances the blood remains infectious for a long period. In Japan it was found that recovered horses remain virulent for as much as four years. A longer incubation period was required when such blood was used for sub inoculations as it gradually lost its virulence with the lapse of time. Van Es claims thirty-five months as the limit of infectivity of recovered horses. A case under observation at the Minnesota Experiment Station still carries virus in virulent form nearly six years after recovery. It may also happen that the infected animal does not show any obvious symptoms of the disease, so mild is the attack. These virus carriers are a source of considerable danger in any region where attempts are made to control the disease.

POSSIBLE MODES OF TRANSMISSION OF VIRUS

The method by which the virus is transmitted from animal to animal is one of prime importance and has been the subject of considerable investigation. Various methods have been suggested. Horses kept in the stable side by side for months may never contract the infection one from the other. In the pasture, however, it is usually able to spread readily, altho some observers claim that it is non-contagious even there. The suggestion that it may be conveyed in water and food seems untenable owing to the destructive effect which

sunlight has upon the virus. It has also been found difficult to give the infection thru the digestive tract, even when very large quantities of virulent blood or urine were fed. The droppings and saliva do not contain the virus, but the urine does. Soiled grass or water would hold only a relatively small quantity of urine. In the stable the virulent secretions would be more cumulative and not subject to the destructive action of the sunlight, yet transmission in the stable is more difficult than in the open. Even the fact that the urine is infectious is, however, disputed by many workers. Vallée and Carré in France, Van Es in North Dakota, the Japanese Commission, and Theiler in South Africa make positive claims. Swingle in Wyoming had negative results to all of his investigations on this question. The positive side, however, agrees that large quantities must be fed to produce the disease.

With these facts in mind it would seem that infection with swamp fever thru the alimentary canal was, at least, not the usual method. There is nothing to indicate infection thru the respiratory passages. The only other method by which the virus could gain entrance to the body would be thru the skin. For virulent blood to enter thru abrasions of the skin would be rare. For this reason blood sucking arthropods have been suspected for some time and some investigations have been carried out, none of which, however, have seemed very conclusive.

REVIEW OF PREVIOUS INVESTIGATIONS

Francis and Marsteller (1908) record that in Texas they kept a healthy susceptible horse all summer and fall in the same pasture with infected horses and never obtained a transmission, altho many varieties of blood sucking flies were present most of the time.

Several observations on the insect transmission of swamp fever of horses were made by the commission appointed by the Japanese government in 1909 to investigate this subject. They noticed that the disease seemed to be easily transmitted in the field but not so in the stable, and drew the conclusion that insects must be the transmitting agent. To test this observation they erected three enclosures each large enough to hold several animals comfortably. One was enclosed with wire screening, the others were simply enclosed with a high board fence. The fenced enclosures were 12.7 meters apart. In the screened enclosure were placed both infected and noninfected horses. In one of the fenced areas were placed nine infected horses and in the other four healthy horses. Some of the noninfected horses in the screened enclosure contracted swamp fever, but only after very long incubation periods, in one case of six months. Those in the fenced enclosures showed the first symptoms of the disease one month after exposure.

They had not come into contact with infected horses, but blood sucking insects had free access. The test was repeated with very similar results, and a third repetition gave still more conclusive results. In each case the experiment ran thruout the entire summer. Their contention was that these results prove that the disease is easily transferred when animals are not in contact but when free access for insects is possible. A shorter incubation period follows than when infection takes place in the stable and the infection is as virulent as in mixed pasture infections. Consequently, they conclude that transmission of the disease is accomplished only thru the agency of insects which are capable of flying.

The Japanese Commission gave very careful consideration to the various blood sucking flies and tried experimnets with some, but in their report do not give any details of how the experiments were carried out. Mosquitoes they claimed were present only in the stables and as stable transmission was rare had to be excluded from consideration. At the time when Simulium "monopolized the pasture," no infections occurred. Ticks were excluded because of their inability to move over long distances. *Stomoxys calcitrans* was abundant and there was every chance for it to act as the carrying agent, but they felt sure that it was not. They tried to feed the flies on the horses by hand, but negative results followed. Many flies were kept in the screened enclosure where there were confined healthy and infected horses. One healthy horse developed a doubtful case after an incubation period of six months, so they excluded Stomoxys from consideration. Tabanids were thought to be the guilty agent, because of their great numbers, violent attacks upon the horses, and because the seasonal appearance of the disease coincides in Japan with the appearance of the flies. They were, however, unable to handle the flies successfully, so no experiment was tried.

Bots (*Gastrophilus intestinalis*) were also taken into consideration, because of the possibility of the larvae taking up the virus and passing it on thru the adult and egg to the next generation. Several bots were taken from a patient which had died of the disease, crushed, filtered and injected into healthy animals, but results were negative. The next season they freed all their horses from bots before putting them into the pastures, but it had no influence upon the results. K. R. and R. Seyderhelm (1914) took up this line of investigation in Germany.

In Wyoming, Swingle (1912) tested the ability of certain tabanids to act as carriers. He used a large screened cage capable of holding three horses. Large numbers of tabanids (spp. not given) from time to time were let into the cage with a sick horse. He experienced considerable difficulty in keeping them alive in the cage and in getting them to feed, but at one time fifty and at another sixteen and lesser

numbers at other times were observed to bite the horse. He endeavored to persuade these flies to feed on a second susceptible horse, but failed with the exception of one fly. While his experiments gave negative results, Swingle suggested a way in which tabanids might act as transmitters of swamp fever. He noticed that while feeding many flies voided fresh blood onto the skin of the horse, and that this apparently undigested blood was voided as long as three days after feeding. He thought that such blood voided onto grass might be eaten by horses and infection acquired. The difficulty of securing infection thru the digestive tract even with large quantities of infective blood would seem to preclude such a probability. There is a possibility, however, that this voided blood might enter the wound made by the proboscis and mechanical transmission be produced in this way as well as by blood on the mouth parts.

The investigations in Wyoming were continued by Scott (1914). He used a cage large enough to accommodate five horses. Adult mosquitoes, species not given, were collected and placed in the cage. Some "wild flies" (tabanids) were unavoidably brought in from the swamps with the mosquitoes, but died in the cage quickly. House flies and Stomoxys, however, survived and bred in the cage. During July the mosquitoes were most abundant. In July a sick horse was alternated with well horses, each being left in the cage for several days at a time. Mosquitoes were repeatedly observed to feed upon the horses, but the results were negative. During August when Stomoxys and *Musca domestica* were more abundant in the cage the experiment was repeated and Stomoxys was seen to bite the horses several times. One of the three healthy horses used, showed a rise of temperature, but did not react again. A second horse, after three recurrences of the temperature rise, died on October 5, and subinoculation of the blood produced typical cases. The third horse gave no reaction. These results do not, however, seem to us to be conclusive. In the first place there were several varieties of flies in the cage; during the second part of the experiment the healthy horses employed were the same as those which had been exposed to the mosquitoe bites during the previous month, and then there seemed to have been no provision made to prevent infection by contact during the latter part of the test, as the healthy horses were kept in the cage during the entire course of the experiment, even when the infected horses were present.

A third variation of this test was carried out, by transferring flies from the first cage where they had fed on infected horses, to a second new cage where were one survivor of experiment one and two other healthy horses. The results were negative. Scott thought the reason was that the nights at that time in August and September were very cold, frost occurring several times.

Further experiments have been carried out by Scott in Wyoming with mosquitoes, Stomoxys, and tabanids, but the results have not yet been published. He claims that *Stomoxys calcitrans* is the carrier in Wyoming.

EXPERIMENTAL WORK

An extensive study of swamp fever has been made at the Minnesota Experiment station by Dr. C. F. Flocken, who was stationed there for that purpose by the U. S. Bureau of Animal Industry. In the spring of 1915 work was begun by the writer on the insect transmission of the disease, in cooperation with the Bureau.

A careful survey was first made to ascertain exactly what biting insects or insect allies were present where the disease was found and whether or not they fulfilled the following required conditions to make them possible transmitting agents. Such an agent must be found over a very wide area; it must be a ready feeder on the horse and be able to pass rapidly from one to another. The fact also that swamp fever in Minnesota usually begins to appear in July, increasing in August, the incubation period being from ten to thirty days, made it appear that the early spring breeding forms were more likely to be the carriers.

Ticks are represented in Minnesota mainly by *Dermacentor variabilis,* which does not attack the horse extensively and is not found abundantly in all localities, so that it can be eliminated. Species of Simulium are present and appear early in the season, but not so early as the tabanids, and are not present in all parts of the state. *Chrysops moerens, C. striatus,* and *C. cuclux* are quite common over most of the state but appear only in late July and August.

Mosquitoes are present everywhere. The early spring breeders such as *Aedes fuscus, A. auroides, A. canadensis* and *A. sylvestris* are especially abundant in our swamp fever areas, but other species are plantiful thruout the summer and autumn. They will be found inside of stables as well as in pastures, but stable infection seems to be rare. If mosquitoes were the agent of transmission swamp fever would probably be more widely epidemic. The same can be said for *Stomoxys calcitrans,* except that it is not abundant in early summer and in that respect does not coincide with the requirements set for possible carriers. It is also found in stables.

Several species of Tabanus appear in spring and early summer, during open seasons coming as early as late May, but never later than early July. The early spring forms are *T. lasiophthalmus, T. affinis, T. zonalis, T. illotus* and *T. septem trionalis.*

Other forms appear during mid and late summer, such as *T. longitudinalis* and *T. atratus,* but in very small numbers. These early tabanids are the worst insect pest of horses and cattle in the northern

half of the state, where much of the land is still virgin timber and muskeg, or at least still undrained and swampy. These seemed to fulfill the conditions which were sought, and it was decided to begin work with these horse flies.

A cage 12 by 24 feet was constructed and divided into three equal parts. One end apartment was cut off from the rest by a partition of fine wire netting, insect proof and intended for a check horse, if desired. The other two parts were separated only by a double partition of half inch mesh wire, to keep animals from coming into contact, but to allow of free movement for insects. The roof was covered with screening and the floors were cement, so that the enclosure could be kept perfectly clean. Two screened vestibules, of a size to allow the passage of a horse gave admission to the two parts of the cage. This cage was constructed on one side of a low one story building containing a series of stables, one of which opened into each part of the cage, so that both animals and insects could seek shelter inside if they wished.

It was impossible to breed flies for the work, but it seemed feasible to collect them and place them in the cages. Altho a journey of 100 to 150 miles had to be made for each lot, it was possible to get a large quantity of flies safely to the laboratory. They were usually collected during the late afternoon from cattle or in stables. From 300 to 500 were placed in each traveling cage. Dilute honey and water was provided as food. The collector chose night trains, if possible, in order to avoid the excessive heat of day trains at that time of year. Consequently, half to three-quarters of the flies were active upon arrival at the University Farm. The first lot of flies let loose in the cage perished by beating themselves to death on the netting. After this we resorted to hand feeding. The flies were kept in an outdoor insect breeding cage, 4 by 4 by 6 feet, supplied with a pan of water and grass, and a small tree. Each day these were removed and fed on the horses. Even with this arrangement only three lived long enough to feed more than once, one of these three times, and the other two twice. They would, as a rule, however, feed readily on syrup or dilute honey, but all died in a few days.

In the known cases of disease transmission by tabanids it occurs in a purely mechanical way. This fact was kept in mind. Horse 25 was the source of virus; he had had a severe attack of the disease four years previously and still carried virulent virus; in fact, three months after this experiment, blood from this horse reacted promptly on injection into another horse. Horse 32 was healthy. Flies were allowed to feed on Horse 25 for about one minute, then removed and transferred quickly, with only five to ten seconds interval to Horse 32, where they finished feeding. The process required, altogether, three to five minutes. Hand feeding was difficult, only a comparatively few being

willing to feed under a glass cylinder. Sometimes gentle manipulation of the fly's head with the finger would suffice to give the start. In all, 69 *T. lasiophthalmus* and 2 *T. affinis* fed in this way. Feeding of the flies began on June 26 and ended July 12. Horse 32 was left until October 2, during which period no rise in temperature was noted. On the latter date it was inoculated with active virus and reacted in eleven days, dying on October 29, thus showing that the flies had not carried virus to this horse.

Objections may be raised to this experiment on the grounds that Horse 25 did not have an acute form of swamp fever during the course of the experiment, and that the virus in the blood was not sufficiently active for the small quantities carried by the flies to cause any reaction. However, in nature there must be many such cases responsible for the spread of the disease, and it would seldom happen that as many as seventy-one flies would complete an interrupted feeding on two horses. Then the blood proved to be active upon subinoculation into another horse after the experiment was over.

It was intended to duplicate this experiment during the spring of 1916, using a newer case for the reservoir. A succession of unusual seasons, however, had reduced the number of horse flies so as to make it impractical to secure a sufficient quantity for the purpose. Consequently, it was necessary to work during the past season upon the insect which we considered as second choice, i. e., *Stomoxys calcitrans*. This fly is present everywhere in the state from early July until cold weather, increasing as the season progresses. They are abundant about the University Farm, and altho swamp fever cases have been kept there for several years in stables and in pastures no case of transmission has occurred which could by any manner of reasoning be laid at the door of Stomoxys. As stated previously, if it was the common carrier of the disease, widespread epidemics might be expected to occur.

Stomoxys was bred in large quantities until a dry period came on in late July lasting thru August. The breeding jars were quickly depleted by this sudden excessive heat and wild flies began to become scarce also. For this reason the last lot of flies used were wild flies captured in the dairy barn.

The first attempts to feed Stomoxys by hand were so discouraging that this method was abandoned. They refused to feed when placed in large test tubes, lamp chimneys and wire gauze cylinders; but as soon as let free in the cage went to the animal at once and fed eagerly. Flies were, therefore, liberated in the large cage and allowed to feed at will. Two phases of the experiment were run simultanously. In the small isolated cage the horse acting as reservoir of the virus and the healthy horse were alternated, leaving each one in for one hour until

the flies had been able to feed, next morning the other horse was left for an hour, etc. This cage was called Cage A.

In Cage B, where the horses were kept separate by a large mesh double wire partition, the flies were allowed to feed at will. The same horses were used in this cage, being returned there each morning after the hour in Cage A. When a fresh lot of flies was let into this cage the horses were tied close to the double partition and two attendants gently brushed them back and forth thru this partition so as to make doubly sure of the possibility of mechanical transmission. During the last few days of feeding the flies the horses were tied close together in one cage, their heads being carefully covered and other precautions being taken to prevent possibility of actual contact and flies fed and brushed back and forth from one horse to the other until they had become full fed.

Horse 25 was again used a source of virus, as his blood had been proved virulent during the previous winter by subinoculations. For part of the experiment Horse 36 was also used. This was a virulent case brought in the previous September. During the course of the experiment he succumbed to the disease. Horse 38 was the healthy horse.

The feeding of the flies began on July 20 and ended August 31. In Cage A, where it was hoped opportunity would be given for biological transmission, a total of 1,425 flies were used. In Cage B, the double cage, 2,800 flies were used. The flies invariably fed well, but could not be made to survive more than about one week on the average, altho every detail to aid their survival was supplied. Probably the unusual hot, dry weather hastened their death, as it was doing with the wild flies.

Horse 38 remained normal until October 14, when it fell and dislocated its shoulder, making it necessary to kill it. The incubation period in a case like this might last for three months so that the results here are not as definite as were desired. Blood was taken from the horse before death, however, and inoculated into Horse 42. Horse 42 showed definite febrile reactions on November 6 and 7, December 10 and 11 and December 17, 18 and 19; the high temperature continuing more or less marked from the last date until its death on January 11, 1917.

Blood from Horse 42 was filtered and injected into Horse 43 on February 24. This horse showed typical febrile reactions on March 10 and 11, April 10, 11 and 12, April 29 and 30, May 1 and 2, May 23 to 26. On the last date the horse was down and was killed.

From this experiment it seems probable that swamp fever of horses *can* be carried from one horse to another by the biting stable fly, *Stomoxys calcitrans.*

The results of these experiments, together with a study of the investigations of other workers has not, however, fully convinced us that insects are the usual or only carriers of the disease.

Important References

Francis, M., and Marsteller, R. P. 1908. Infectious Anemia of the Horse. Texas Agr. Exp. Sta. Bull. 119.

Japanese Commission. 1914. Report on the Results Obtained by the Special Committee for the Investigation of Infectious Anemia Among Horses. The Horse Administration Bureau, Tokyo.

Scott, John W. 1914. The Transmission of Swamp Fever. Ann. Rept. Wyo. Agr. Exp. Sta., *23*: 180-188.

1915. Insect Transmission of Swamp Fever. Science, n. s., *42*: 659.

Seyderhelm, K. R., and R. 1914. Die Ursache der perniziösen Anämie der Pferde. Arch. exper. Path. u. Pharmakol., *76*: 149-201.

Swingle, Leroy D. 1913. Transmission of Swamp Fever in Horses. Ann. Rept. Wyo. Agr. Exp. Sta., *23*: 93-123.

Van Es, L., Harris, E. D., and Schalk, A. F. 1911. Swamp Fever in Horses. No. Dak. Agr. Exp. Sta. Bull. 94.

University Farm, St. Paul, Minn.